Molecular Pathways and Targeted Therapy in Cholangiocarcinoma

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Abstract: Cholangiocarcinoma (CCA) encompasses a rare group of malignancies arising from epithelial cells lining the biliary tree that connects the liver and gallbladder to the small intestine. Most patients present with advanced incurable disease that has a poor prognosis, and standard treatment options remain limited. Effective nontoxic treatment options for advanced CCA are needed. Fibroblast growth factors (FGFs) and their fibroblast growth factor receptor (FGFR) pathways are crucial to cellular proliferation, cellular survival, and differentiation of many malignancies, but are especially relevant in CCA. The targeting of FGF/FGFR has become the most promising approach to treating patients with advanced/metastatic CCA. Here we review CCA, and discuss the promise of FGFR-directed therapy in advanced CCA.

Introduction

Cholangiocarcinoma (CCA) encompasses a rare group of malignancies arising from epithelial cells that line the biliary tree. It is associated with a poor prognosis and has limited standard treatment options. CCA is the most common primary biliary tract malignancy and the second most common primary hepatic malignancy,[1] affecting 2000 to 3000 people each year in the United States.[2]

More than 90% of CCAs are adenocarcinoma and are divided into histologic types based on their growth patterns: mass forming, periductal infiltrating, and intraductal growing.[3] CCA is typically classified as either intrahepatic or extrahepatic. It generally arises de novo, without associated specific inherited risk factors. Several nongenetic risk factors for intrahepatic CCA have been identified, including liver cirrhosis and chronic viral hepatitis B and C.[4] The contribution of hepatitis B and C to tumor development differs by geography. Hepatitis B is endemic in Asian countries, and hepatitis C is more prevalent in Western countries.[4] Southeast Asia has a very high incidence of CCA that stems from the high prevalence of hepatobiliary flukes that infect humans, specifically *Opisthorchis viverrini* and *Clonorchis sinensis*.[4] A well-established association exists between primary sclerosing cholangitis, which is marked by chronic

inflammation with liver injury and likely proliferation of the progenitor cells, and perihilar CCA.[4]

CCA has an aggressive natural course, with a median overall survival of less than 24 months.[5] Surgical resection and liver transplant are the only curative therapeutic modalities,[6] and are reserved for patients with early-stage disease. No curative medical therapies currently exist for advanced CCA. Cytotoxic combination chemotherapy with gemcitabine and cisplatin has become the standard treatment for patients with advanced or metastatic disease. The fact that responses to chemotherapy typically are limited and median overall survival is dismal[3] has made enhanced understanding of molecular pathogenesis of CCA imperative.

In recent years, the advent of next-generation sequencing technology has substantially improved the ability of scientists to understand the complex molecular events occurring in CCA, including the interactions between gene mutations and disease risk factors. Among the discoveries regarding the important mutations associated with the pathogenesis of CCA are mutations in *IDH1/2*, as well as mutations in the genes involved in chromatin remodeling, such as *ARID1A*, *PBRM1*, and *BAP1*.[7] Deregulation of several growth factor tyrosine kinases, noted in various malignancies including CCA, plays a critical role in tumor initiation and progression.[8] These include the fibroblast growth factor receptor (FGFR) pathway and the ERBB family of receptor tyrosine kinases, including epidermal growth factor receptor (EGFR) and hepatocyte growth factor receptor (Table 1).[8-10] The most promising target for CCA identified in recent years is within the fibroblast growth factor (FGF) signaling pathway.

FGFR Signaling Pathway

The FGF pathway consists of 22 human FGFs and 4 transmembrane receptor tyrosine kinases, the FGFRs 1 through 4.[11] The *FGFR* fusion has been noted exclusively in intrahepatic cholangiocarcinoma (ICA).[8] The ubiquitous role of FGF signaling in various biological processes is integral to cell survival and increases susceptibility to oncogenic transformation with aberrant FGF signaling.[12] Deregulated FGF signaling mediates carcinogenesis by enhancing cellular proliferation, migration, survival, and invasion and promoting tumor angiogenesis.[12] Receptor overexpression results in gene amplifications or changes in post-transcriptional processing; point mutations may result in constitutive receptor activation or decreased sensitivity to ligand binding; translocation can produce fusion proteins with constitutive activity; and isoform switching and alternative splicing can reduce specificity to FGFs.[13] *FGFR1* amplification was the most common abnormality within the overall spectrum of FGFR anomalies; notably,

Table 1. Molecular Pathways in Cholangiocarcinoma and Available Inhibitors

Signaling Pathway	Mutations	Prevalence in CCA, percentage	Therapeutic Agents
Tyrosine kinase	*EGFR* *HER2*	10-15 5-20	Erlotinib Gefitinib Trastuzumab Pertuzumab Lapatinib
Tyrosine kinase	*FGFR*	10-15	Ponatinib
Tyrosine kinase	*ROS1*	1-9	Crizotinib
Tyrosine kinase	*MET*	5	Crizotinib Cabozantinib Tivozanib LY2801653 Onartuzumab
Tyrosine kinase	*VEGFR*	50-60	Bevacizumab
RAS/RAF/ MEK/ERK	*KRAS* *NRAS* *MEK1/2*	15-25	Selumetinib Trametinib Refametinib Binimetinib
RAS/RAF/ MEK/ERK	*BRAF*	5	Vemurafenib Panitumumab Irinotecan PLX8394 Regorafenib Sorafenib
PI3K/AKT/ mTOR	*PI3K* *PTEN*	4-25	Apitolisib Copanlisib Everolimus Temsirolimus Sirolimus MK-2206 Buparlisib
Glucose metabolism enzyme	*IDH1* *IDH2*	10-15	Enasidenib Ivosidenib AG-121 AG-881
JAK/STAT	*JAK1/2* *TYK2* *STAT3/5*	50 in ICA	Ruxolitinib OPB-31121 Amcasertib Napabucasin
Hedgehog	*GLI1* *PTCH1*	30-50[a]	BMS-833923 Vismodegib
Somatic mutation	*BAP1*	25	Vorinostat Panobinostat

CCA, cholangiocarcinoma; ICA, intrahepatic cholangiocarcinoma.
[a] Small sample (50 patients).

FGFR4 was also seen to have high rates of amplification.[14] Gene mutations and arrangements affecting FGF/FGFR signaling were less common than amplifications.[14] Of the 4 FGFR receptors, *FGFR2* and *FGFR3* are identified as having comparatively more frequent gene rearrangements or fusions.[14]

Researchers from the Mayo Clinic found that 13 of the 156 evaluated biliary tumors harbored an *FGFR2* translocation.[15] Twelve of those were among the 96 patients with ICA, accounting for a prevalence of 13% among this cohort.[15] Survival of CCA patients with *FGFR2* fusions was significantly higher than those without *FGFR2* fusions (123 vs 37 months), suggesting the potential utility of *FGFR2* fusion identification as a prognostic marker.[15] *KRAS* and *BRAF* mutations were not present in CCA patients with *FGFR2* translocations, signifying the potential of these gene fusions as driver mutations.[8] The anatomic restriction not only is suggestive of differing genomic causes of CCA based on primary site of origin, but also points to the possibility that CCA may arise in response to differing exposures, including viruses such as hepatitis B and C and environmental toxins with predilections for liver injury.[6] In that same study from the Mayo Clinic, CCA patients with *FGFR2* fusions were younger (median age, 52 vs 65 years) and more likely to be female than male (13% vs 4%, respectively).[15] Similar findings were reported by researchers from Japan's National Cancer Center Research Institute, in a study consisting of 102 patients with CCA. Sixty-six of the patients had ICA, and 36 had extrahepatic carcinoma. Of the 66 patients with ICA, 9 (13.6%) were found to have *FGFR2* fusions. No survival or sex differences were reported in this study.[16] *FGFR2* translocations were more likely to be noted in the patients with de novo CCA rather than those with preexisting primary sclerosing cholangitis.[15] The *FGFR2* fusions were found in patients with nonadvanced, early-stage CCA following surgical resection. This suggests that *FGFR2* fusion may be an early oncogenic event that serves as a driver of CCA, and would be present in a substantial proportion, or the majority, of tumor cells.[6] Recent studies clearly have demonstrated that *FGFR2* fusions may have both predictive and prognostic implications in CCA.

Current science and the application of molecular profiling have further elucidated other important drivers in the pathogenesis of CCA. *IDH1/2* mutations are found in 10% to 23% of patients with ICA.[7] Having an *IDH1* mutation is associated with poor prognosis in patients with extrahepatic carcinoma.[7] Patients with *IDH1/2* mutations have significant overexpression of *FGFR2*, *FGFR3*, and *FGFR4* in the absence of *FGFR* mutations or *FGFR2* fusions.[17] The basis for the association is unknown, and having a better understanding would be useful.[17] Because of the importance and prevalence of the *FGFR2* fusion

and *IDH1* and *IDH2* mutations, these mutations have become the focus for targetable pathways for drug development in advanced CCA.[4]

Among all of the potentially targetable driver mutations specifically found in ICA, the most frequent are mutations that alter FGF signaling, primarily by *FGFR2* fusions. These mutations occurred in up to 45% of the 107 patients with ICA in a study by Sia and colleagues.[18] FGF downstream signaling pathways that become activated by FGF fusion proteins include the RAS/RAF/MEK/MAPK axis.[19] A number of studies have recently reported new *FGFR2* fusions (*FGFR2-KCTD1*, *FGFR2-TXLNA*, *FGFR2-PPHLN1*), further underlining the importance of the pathway in the carcinogenesis of ICA.[18] Identifying fusion subgroups will allow for selective targeting and novel therapeutic opportunities. Current therapeutic options include nonselective and selective FGFR inhibitors.

Nonselective FGFR Inhibitors

Nonselective FGFR tyrosine kinase inhibitors (TKIs) are compounds that bind to relatively conserved ATP-binding domain in receptor tyrosine kinase.[20] Nonselective FGFR TKIs target other receptor tyrosine kinases, such as vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor receptors (PDGFRs), and usually produce modest bioactivity against the FGFR family. Dual inhibition with VEGFRs/PDGFRs has the potential benefit of simultaneously targeting angiogenesis and tumor cell proliferation. But these agents are less potent against the FGFR signaling pathways, and give rise to a variety of toxic side effects that limit their ability to be administered at doses required for FGFR inhibition.[20] Pazopanib (Votrient, Novartis) and ponatinib (Iclusig, Ariad) have demonstrated activity in individual patients with advanced ICA and *FGFR* fusions or mutations.[21]

Pazopanib was first investigated in CCA in 2011. Pazopanib is an orally available multikinase inhibitor of VEGFR, PDGFR, KIT, and FGFR, as well as RAF, that is approved by the US Food and Drug Administration (FDA) for advanced renal cell carcinoma and advanced refractory soft tissue sarcoma (Table 2).[22] Trametinib (Mekinist, Novartis) is an orally available highly specific inhibitor of MAP kinase/ERK kinase 1 (MEK1) and MEK2 that is FDA-approved for *BRAF* V600E– and V600K–mutated unresectable or metastatic melanoma.[23] Together, pazopanib and trametinib provide vertical inhibition of RAF and MEK, which causes a synergistic inhibitory effect. This combination targets both VEGFR and PDGFR, which is theorized to generate potent inhibition of tumor angiogenesis.[24] Shroff and colleagues from the University of Texas MD Anderson Cancer Center and

Table 2. Current FGFR Targeted Therapy in Cholangiocarcinoma

Agent	Target	Toxicity	Trials	FDA Approval	FDA Indications
Nonselective Inhibitor					
Pazopanib (in combination with trametinib)	FGFR1-4, VEGFR, PDGFR, KIT (BRAF, MEK)	Rash, hypertension, nausea or vomiting, fatigue, diarrhea, thrombocytopenia	Phase 3	Yes	Renal cell carcinoma, soft tissue sarcoma
Ponatinib	FGFR1-4, *BCR-ABL*, VEGFR, PDGFR, SRC, KIT, RET	Hypertension, rash, fatigue, dry skin, headaches, arterial/venous thrombosis, constipation, arthralgia, peripheral vascular disease	Phase 3	Yes	CML, Ph+ ALL
Selective Inhibitor					
Infigratinib	FGFR1-4	Hyperphosphatemia, fatigue, constipation, stomatitis	Phase 3	No	
Erdafitinib	FGFR1-4	Hyperphosphatemia, dry mouth, stomatitis, dry skin, hand-foot syndrome	Phase 3	Yes	Metastatic bladder
TAS-120	FGFR1-4	Hyperphosphatemia, transaminitis, dry skin, diarrhea, dry mouth	Phase 1/2	No	
Pemigatinib	FGFR1-3	Hyperphosphatemia, alopecia, diarrhea, fatigue, decreased appetite	Phase 3	No	
Debio 1347	FGFR1-3	Hyperphosphatemia, fatigue, diarrhea, nausea, decreased appetite	Phase 1/2	No	
FGF Ligand Traps					
FP-1039	FGF1, FGF2, FGF4	Diarrhea, fatigue, nausea	Phase 1	No	
Anti-FGFR Monoclonal Antibodies					
Bemarituzumab, also known as FPA144	FGFR2b	Upper respiratory infection, alopecia, fatigue	Phase 1/3	No	

ALL, acute lymphocytic leukemia; CML, chronic myeloid leukemia; FDA, US Food and Drug Administration; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; Ph+, Philadelphia chromosome–positive; VEGFR, vascular endothelial growth factor receptor.

Johns Hopkins evaluated 25 patients with advanced CCA who were treated with pazopanib plus trametinib.[24] No patient had received prior therapy with a MEK inhibitor, and all patients had received a median of 2 prior systemic therapies. Twenty patients had perihilar or distal CCA, and 5 patients had ICA. Twenty (80%) were evaluated for objective response rate (ORR) and disease control rate (DCR) by Response Evaluation Criteria of Solid Tumors (RECIST 1.0). The ORR was 5% (95% CI, 0.13%-24.9%) and the DCR was 75% (95% CI, 51%-91%). The median progression-free survival (PFS) was 3.6 months.[24] The median overall survival (OS) was 6.4 months. The OS rates at 2, 4, and 6 months were 88%, 76%, and 52%, respectively.[24] Treatment-related toxicities were predominantly of mild or moderate severity, with the most common events including rash (80% of patients), hypertension (64%), nausea or vomiting (64%), fatigue (60%), diarrhea (52%), and thrombocytopenia (40%). No treatment-related deaths occurred.[24]

Ponatinib is an orally available, nonselective FGFR inhibitor. Specifically, it is a multitargeted TKI of BCR-ABL, VEGFR, PDGFR, SRC, KIT, and RET. It has FDA approval for the treatment of patients with chronic myeloid leukemia or Philadelphia chromosome–positive acute lymphocytic leukemia (Table 2). Ponatinib can inhibit the enzymes needed for cell growth and induces

tumor necrosis in patients with advanced ICA and *FGFR2-MGEA5* fusion.[21] Currently, ponatinib is being examined in a phase 2 clinical trial of all advanced biliary cancers (including CCA) with fusions in *FGFR2*.[25] Twelve patients are enrolled, and are being treated with 45 mg daily.[25] This trial is still active, but is not recruiting. The dose-limiting effect on FGFR inhibition by nonselective inhibitors has led to the investigation of selective FGFR inhibitors.

Selective FGFR Inhibitors

Selective FGFR inhibitors are highly selective and highly bioactive against FGFR.[20] They are associated with an FGFR-specific toxicity profile that includes hyperphosphatemia, gastrointestinal toxicity, and cutaneous toxicity. The hyperphosphatemia, which was caused by the inhibition of FGFR1, was found to be manageable.

Infigratinib (BGJ398) is an orally bioavailable, selective, ATP-competitive pan-FGFR kinase inhibitor that has activity against tumor models harboring *FGFR* alterations.[26] The first clinical trials of infigratinib were in adults with advanced solid malignancies, and the agent showed antitumor activity and acceptable side effects in a phase 1 clinical trial of 132 patients with solid organ tumors and known *FGFR* mutations.[19] The follow-up phase 2 clinical trial was designed to evaluate infigratinib in 71 patients with advanced CCA and *FGFR* mutations whose disease had progressed on platinum-based chemotherapy.[27] All patients were positive for *FGFR2* translocation, and 5 patients had *FGFR2* mutations.[28] The median duration of therapy with infigratinib was 5.5 months, and the median duration of follow-up was 8.4 months.[28] Sixty-two patients discontinued treatment. The DCR was 83.6%, with a median PFS of 6.8 months and a median duration of disease control of 5.4 months. The ORR and DCR among patients with tumors bearing *FGFR2* fusions were 26.9% and 83.6%, respectively. Infigratinib was administered at 125 mg once daily on a 3-weeks-on/1-week-off schedule, and demonstrated an ORR of 39.3% among patients who had received at least 1 prior line of treatment and 17.9% among patients who had received 2 prior lines of treatment.[28] Infigratinib is the first in this class of FGFR kinase inhibitors with manageable toxicity to show meaningful clinical activity against chemotherapy-refractory CCA that contains an *FGFR2* fusion.

Erdafitinib is a second pan-FGFR small-molecule kinase inhibitor that is currently undergoing investigation in clinical settings.[29] In a phase 2 trial with advanced solid tumors for which standard curative treatment is no longer effective, erdafitinib showed antitumor activity only in the 23 patients with *FGFR* mutations, whereas 36 patients who did not have confirmed *FGFR* mutations

had no response.[29] Among these 23 response evaluable patients, researchers noted 4 confirmed responses and 1 unconfirmed partial response. Sixteen patients had a stable response, and 8 of these patients had stable disease for more than 3 months. Results from a phase 2a trial were presented at the 2018 European Society for Medical Oncology (ESMO) annual meeting. Of the 193 patients who were molecularly screened, 29 had *FGFR* alterations. Of those with alterations, 7 patients had *FGFR2* fusions, 3 had *FGFR2* mutations, and 2 had *FGFR3* mutations. Eleven of the 12 patients were deemed evaluable for response.[30] Of these 11 patients, 5 confirmed partial responses occurred in those with *FGFR2* alterations (1 *FGFR2* mutation and 4 *FGFR2* fusions). The ORR, DCR, and 9-month PFS rates were 66.7%, 100%, and 49%, respectively. The most common any-grade adverse event associated with treatment was hyperphosphatemia, followed by dry mouth and stomatitis (Table 2).[30] Adverse events that resulted in dose reduction were noted in 5 patients (45.5%), whereas events that led to dose interruptions were observed in all patients (100%). Three patients experienced non–drug-related serious adverse events, and none of these adverse events led to treatment discontinuation or death. This study showed some promising results, and investigators concluded that a larger sample size was needed to further explore safety and effectiveness of erdafitinib in this population.

TAS-120, an irreversible pan-FGFR inhibitor, was evaluated in patients with advanced solid tumors, who were previously treated with chemotherapy or other therapies including other FGFR inhibitors.[31] This is one of the first drugs to be evaluated in patients who previously received FGFR inhibitors; data from this study were presented at the ESMO World Congress on Gastrointestinal Cancer 2018 on 45 patients with CCA harboring *FGF/FGFR* aberrations (*FGFR2* fusions or other *FGF/FGFR* aberrations). Among 28 patients with *FGFR2* fusions, 8 patients had received prior FGFR inhibition. Twenty patients (71%) experienced tumor shrinkage, and 7 patients achieved a confirmed PR. The ORR in these patients was 25%, and 15 patients (54%) experienced stable disease as their best response, with 7 patients still on treatment. Among 17 patients with other *FGF/FGFR* aberrations who received TAS-120, 18% achieved a confirmed PR. Of the 17 patients, 5 had received prior FGFR inhibitors. As with the other selective inhibitors, the most common treatment-related any-grade adverse event in all patients was hyperphosphatemia. TAS-120 showed a clinical benefit rate of 78.6% in patients with CCA and an *FGFR2* fusion. This response was also seen in patients whose disease progressed during treatment with a prior FGFR inhibitor. This is very encouraging because patients receiving second-line treatment traditionally

experienced a 5% rate of tumor shrinkage. Although TAS-120 produced the best response in patients with an *FGFR2* fusion, it also showed activity against other *FGFR* alterations, as well as *FGFR2* resistance mutations. A global phase 2 study called FOENIX-101 (A Study of TAS-120 in Patients With Advanced Solid Tumors; NCT02052778) that involves ICA patients with an *FGFR2* fusion is under way.

Pemigatinib is a potent, selective oral inhibitor of FGFR isoforms 1, 2, and 3 that has demonstrated selective pharmacologic activity in cancer cells with FGFR alterations.[32] The ongoing phase 2 FIGHT-202 trial (Efficacy and Safety of Pemigatinib in Subjects With Advanced/Metastatic or Surgically Unresectable Cholangiocarcinoma Who Failed Previous Therapy) is evaluating the use of pemigatinib in patients with advanced, metastatic, or surgically unresectable CCA whose disease failed to respond to at least 1 previous treatment.[32] In patients with *FGFR* translocations who were followed for at least 8 months, interim study results demonstrated an ORR of 40% (the primary endpoint) and a median PFS of 9.2 months (a key secondary endpoint). Forty-seven patients were evaluated for response. The combined ORR was 40%, including 19 (40%) patients with confirmed partial responses and 21 (45%) patients with stable disease. The combined DCR was 85% (40/47). The median PFS was 9.2 months and the median OS was 15.8 months. The side effect profile is similar to that of the other nonselective inhibitors (Table 2).

A second-generation FGFR selective inhibitor, Debio 1347, was developed with a different chemical scaffold than the other selective inhibitors.[33] Debio 1347 is an orally available, highly selective pan-FGFR inhibitor with a potent antitumor effect in preclinical models bearing genetic alterations in *FGFR1-3*.[33] In a phase 1 study of 56 patients, including patients with *FGFR* mutations (n=17), amplifications (n=28), and fusions (n=11), 54 patients had evaluable responses. Four confirmed responses and 1 unconfirmed partial response were observed in patients with CCA (*FGFR2* mutation), uterine cancer (*FGFR2* and *FGFR1* amplification), colon cancer (*FGFR2* fusion), and urothelial cancer (*FGFR3* fusion); an additional 10 patients had target regression of less than 30%. Because of the different chemical scaffold, Debio 1347 has shown efficacy against cells harboring an *FGFR2* fusion with the V564F gatekeeper mutation.

Indirectly Targeting FGFR

FGFR-targeted therapies are not limited to the tyrosine kinase domain only. Multiple agents targeting the extracellular domains of FGFR are being investigated. These include isoform-selective inhibition and the FGF ligand trap.

Bemarituzumab (FPA144) is a humanized monoclonal antibody directed against the FGFR2b isoform.[8] It is an FGFR2-IIIb–blocking monoclonal antibody that has been shown to inhibit the growth of *FGFR2*-amplified gastric cancer xenografts by 72% to 100%.[34] Data from 13 patients enrolled in an early trial showed that no dose-limiting toxic effects were associated with bemarituzumab administration. Upper respiratory infection, alopecia, and fatigue were the adverse events reported in more than 1 patient.[35] Prior studies have shown that FGFR2-IIIb exhibits selectivity in binding to the FGF7 and FGF10 ligands.[6] This is an attractive therapeutic option because it could potentially avoid the off-target toxicities of FGFR small-molecule kinase inhibitors (SMKIs).[36] The goal is to reduce the potential toxicity of the pan-FGFR inhibition given the specificity of antibody-antigen interactions.[37] FGFR2-IIIb antibodies could also be positioned in combination with an FGFR SMKI to achieve more complete blockade of the FGFR2 signaling axis.[36]

Another approach to FGFR inhibition that is being evaluated is FGF ligand traps. The FGF/FGFR signaling pathway impedes ligand binding to the receptors by developing FGF ligand traps.[20] FGF ligand traps sequester FGF ligands, blocking their ability to bind to and activate FGFRs.[20] FP-1039 is a soluble fusion protein consisting of the extracellular domain of FGFR1c fused to the Fc region of immunoglobulin G1 that prevents binding of FGF1, FGF2, and FGF4.[38] It has demonstrated antiangiogenic and antiproliferative properties.[38] In the phase 1 study evaluating FP-1039 in patients with metastatic or locally advanced solid tumors, stable disease was seen in 41.7%.[39] Major adverse events were diarrhea (43.6%), fatigue (43.6%), and nausea (25.6%). No apparent relationship was reported between tumor response and FGF pathway aberrations among the 39 patients enrolled.

Future Directions in FGFR-Directed Therapy

Heat shock protein (HSP) inhibitors are another goal-directed therapy that is being investigated. HSPs, particularly HSP90 and its co-chaperone CDC37, have been shown to serve as chaperones to a wide array of oncogenic client proteins, including FGFR family membranes.[40] HSP90 regulates the maturation and functional stability of a myriad of cellular proteins, including key regulators of cell proliferation, differentiation, and survival.[41] HSP90 and CDC37 are essential in the stability of many oncogenic proteins.[8] The *FGFR1OP2* gene encodes a protein of unknown function known as FGFR1 oncogene partner 2 (FOP2).[8] HSP90-CDC37 forms a permanent complex with FOP2-FGFR1 that protects the resulting fusion protein from degradation and holds it in a permanently active conformation in a leukemic cell line.[42] Inhibition of HSP90 function also reduces the signaling capacity of

FGFR3 and induces its degradation. The combination of ganetespib, a selective HSP90 inhibitor, and infigratinib produced enhanced efficacy compared with either agent alone.[43] These data suggest that HSP90 inhibition may be an alternative, or potentially complementary, approach to kinase inhibition in fusion-driven malignancies.[43]

A growing challenge of FGFR inhibition efficacy is development of drug resistance. The "gatekeeper" mutations in the ATP binding cleft that induce resistance to FGFR inhibition have been identified preclinically.[44] Gatekeeper mutations that include *FGFR3* V555M, *FGFR1* V561M, and *FGFR2* V564F induce resistance to multiple FGFR inhibitors in vitro.[44] Irreversible covalent FGFR inhibitors that bind FGFRs that have gatekeeper mutations have been developed with the aim of overcoming resistance to selective FGFR inhibitors.[44] Another form of mechanism of resistance is mutation in the tyrosine kinase domain *FGFR2* N550K.[45] A third mechanism of resistance is activation of the ERBB family members that results in switching from dependence on FGFR signaling to ERBB signaling, which can be overcome with combination FGFR and EGFR inhibitors.[45] In ICA, acquired resistance to BGJ398 and Debio 1347 has been observed. Debio 1347 has been noted to overcome the V564F gatekeeper mutation. Reversible inhibitors of FGFR, such as BGJ398 or Debio 1347, appear to be subject to development of resistance, as has been demonstrated in recent studies of resistant patients.[46] Goyal and colleagues participated in a multicenter trial looking at the mechanism by which TAS-120 overcomes resistance to selective ATP-competitive FGFR inhibitors.[47] In this study, genomic characterization of pre- and post-progression circulating tumor DNA and tumor biopsies was conducted in 3 patients with *FGFR2* fusion–positive ICA treated with BGJ398[47] and revealed the emergence of the *FGFR2* V565F gatekeeper mutation in all 3 patients, 2 of whom had additional *FGFR2* kinase domain mutations.[46] This study also evaluated 4 patients who progressed on BGJ398 or Debio 1347 and were subsequently enrolled in a phase 1 trial of TAS-120.[47] All 4 patients experienced benefit from TAS-120. Two patients achieved a partial response and 2 achieved stable disease, with a duration of benefit of 5.1 to 17.2 months.[47] Assessment of circulating tumor DNA suggests that TAS-120 has differential activity against individual *FGFR2* secondary mutations compared with ATP-competitive FGFR inhibitors. TAS-120 has activity against multiple secondary *FGFR2* resistance mutations, which likely accounts for the benefit of TAS-120 in patients whose disease previously progressed on BGJ398 or Debio 1347. Combining TAS-120 with BGJ398 or Debio 1347 can overcome that resistance.[48] TAS-120 covalently binds to a highly conserved P-loop cysteine residue in the ATP pocket of FGFR (c492 in FGFR2-IIIb isoform).[17] TAS-120 resistance may develop via mutation of the P-loop cysteine and/or upregulation by bypass tracks.[48] TAS-120 resistance is currently being investigated.[48]

Future efforts should be directed toward the development of FGFR-specific kinase inhibitors with minimal activity against other kinase inhibitors, such as VEGFR and PDGFR.[8] Gene fusions and mutations in CCA need further investigation. Identification of biomarkers for the selection of patients harboring pertinent genetic aberrations is an essential factor in targeted therapy.[18] Efforts are needed to identify the patients who are most likely to benefit from FGFR inhibitors. Investigations into therapeutic dosing as well as toxicity management are also needed.

Preliminary results from development trials of FGFR inhibitors are very promising, with manageable toxicities and significant antitumor activity observed in molecularly selected populations.[45] Preclinical and early clinical data demonstrated that targeting the FGFR signaling pathway can be a promising therapeutic strategy as both monotherapy and in combination with other agents.

Summary

As the landscape of cancer treatment continues to evolve, understanding and targeting driver mutations will become the cornerstone of cancer treatment. In cancers such as CCA that have a poor prognosis and limited treatment options, molecular targeted therapy is even more important. The therapeutic gain that occurs with inhibition of FGF/FGFR signaling is being investigated through different mechanisms. This includes inhibition of the cytoplasmic tyrosine kinase domain as well as binding the extracellular domain and competing with FGFs. FGF ligand traps are being used by blocking the activity of multiple FGF ligands and receptors. Inhibition of FGFR signaling pathways has demonstrated encouraging clinical activity in CCA. Several pharmacologic agents have been developed for the inhibition of FGFR kinases. Multiple clinical trials are currently under way, and more promising data will soon be available.

Disclosures
Drs Dabney, Khalife, Shahid, and Phan have no disclosures to report.

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